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## The Effect of Environmental Calcium Concentrations on Zebra Mussel (*Dreissena polymorpha*) Shell Composition

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The Effect of Environmental Calcium Concentrations  
on Zebra Mussel (Dreissena polymorpha) Shell Composition.

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Project Title: The Effect of Environmental Calcium  
Concentrations on Zebra Mussel (Dreissena polymorpha)  
Shell Composition.

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### ABSTRACT

The calcium content of zebra mussel shells from eight different natural and experimental waters was analyzed. Large control Lake Superior shells have less calcium compared to large shells from the other Great Lakes. The shells' calcium content was dependent upon the environmental calcium level and the length of the growth period spent at each condition. An inverse relationship exists between the size and calcium content of zebra mussel shells. The calcium compounds ( $\text{CaCO}_3$  or  $\text{CaCl}_2$ ) in experimentally hardened water had no effect on shell composition.

### INTRODUCTION

Presently the spread of zebra mussels (Dreissena polymorpha) is a growing concern in the Great Lakes region. One-year-old zebra mussels were apparently introduced into Lake St. Claire in 1986 in the ballast water of cargo ships from freshwater European ports (Mackie, Gibbons, Muncaster, & Gray; 1989 and O'Neill & MacNeill, 1989) (Figure 1). The first confirmed discovery of zebra mussels in Lake St. Claire was reported in June, 1988 (O'Neill & MacNeill, 1989). By autumn 1989, zebra mussels had colonized nearly every firm substrate in Lake Erie including the beaches and water treatment and industrial water systems along Lake Erie and the surrounding rivers (Snyder, 1989). In August 1989, zebra mussels had invaded some Lake Ontario water treatment plants (Minn. Sea Grant, Dec. 1989). After the initial detection of zebra mussels on navigational buoys in September of 1990, the Wisconsin Sea Grant Institute (1991) reported a "light" zebra mussel infestation in Lake Superior, especially in the Duluth/Superior Harbor. By the

fall of 1990, zebra mussels had been reported in all of the Great Lakes (Snyder, 1989).

Zebra mussels pose a threat of becoming a "biofouler" throughout the Great Lakes (Mackie, Gibbons, Muncaster, & Gray; 1989). The mussels threaten to clog industrial and public intake pipes, negatively impact the Great Lakes fisheries, detract from or lessen the recreational and aesthetic value of shoreline areas, serve as vectors for fish and waterfowl parasites, and displace or disrupt native biota (Mackie, Gibbons, Muncaster, & Gray, 1989; Snyder, 1989). Experts estimate nearly \$4 billion in damage to the Great Lakes in the next ten years due to zebra mussel infestation (Oakes, 1990).

With initial sighting of zebra mussels in water samples and on substrates in Lake Superior, there is a growing concern in the Lake Superior region that the lake will succumb to a large mussel population and the subsequent problems as the other Great Lakes. However, Lake Superior is different from the other Great Lakes chemically and physically. These differences may reduce the threat of zebra mussels in Lake Superior.

Originally, the ability of the zebra mussels to spread into Lake Superior was questioned because of Lake Superior's greater depth and colder waters in comparison to the other Great Lakes (Goodchild, 1990). Egg development in zebra mussels appears to be temperature dependent (Mackie &

Flippance, 1983), and the cold temperatures in Lake Superior could prevent large infestation by impairing mussel development (Goodchild, 1990).

Additionally, Lake Superior's calcium concentration is lower than that of the other Great Lakes. For example, Lake Erie contains approximately 37 ppm of calcium and Lake Michigan 32 ppm in comparison to 13 ppm of calcium in Lake Superior. Other studies suggest that the zebra mussel requires calcium for successful reproduction (12 ppm), egg development, and continued shell formation (30 ppm) (Mackie & Flippance, 1983; Nichols, Unpublished). Water chemistry, in particular water hardness (a measure of calcium and magnesium ion concentrations), is a major factor in governing the distribution and population densities of many aquatic molluscs because calcium is an important component of shells (Dussart, 1976; Mackie & Flippance, 1983; Hunter, 1964; Lee & Wilson, 1974).

Zebra mussels exposed to eight different environmental conditions were analyzed for the effects of environmental calcium concentrations on shell formation. Mussel shells from the low calcium environment of Lake Superior were expected to contain less calcium compared to shells from the other Great Lakes. Additionally, shell size and the length of the growth period at different environmental calcium levels were analyzed for the effects on shell calcium content.

## METHODS

The eight zebra mussel test groups consisted of shells with the following histories:

1. **Small Test St. Claire shells** (from Lake St. Claire in Sept, 1991 with an average length of 1.00 cm. Mussels kept alive for 2.5 months in model St. Claire/Erie water with 35 ppm of calcium from  $\text{CaCO}_3$ .)
2. **Large Test St. Claire shells** (from St. Claire in 1991 with an average length of 2.01 cm and held under same conditions as group 1 shells).
3. **Small Test Lake Erie shells in  $\text{CaCl}_2$**  (from Lake Erie Nov, 1991 with an average length of 0.89 cm. Mussels placed in model Erie/St. Claire water with 35 ppm of calcium from  $\text{CaCl}_2$ ).
4. **Small Control Lake Erie shells** (from Lake Erie in 1992 with an average length of 1.05 cm.).
5. **Large Control Lake Erie shells** (from Lake Erie in 1992 with an average length of 1.74).
6. **Large Control Lake Michigan shells** (from Green Bay in August, 1992 with an average length of 1.30 cm.).
7. **Small Test Lake St. Claire shells in Lake Superior Water** (from Lake St. Claire in Sept, 1991 with an average length of 0.92 cm. Mussels were kept alive for 2.5 months in natural Lake Superior water with a calcium concentration of 13 ppm).
8. **Large Control Lake Superior shells** (from St. Louis Bay-North Channel of the Duluth/Superior Harbor in fall, 1991 with an average length of 1.44 cm.).

The zebra mussels were stored frozen until analysis of the shells could be performed. After the samples thawed, the tissues were removed from the shells with a spatula. The shells were dried over night at room temperature. The maximum lengths of the shells were measured from anterior to posterior end.

All glassware used was acid rinsed to reduce the risk of contamination. After grinding the dry shells with a mortar and pestle, the sample masses were determined on a

Mettler AE 100 electronic balance in the Erlenmeyer flask later used to dissolve the samples. The shell components were initially dissolved with 20 mL of 1.6 M HCl, and evaporated to dryness on a hot plate. The residue was redissolved in 5 mL of 0.3 M HCl, diluted to about 100 mL with distilled water, and digested over low heat for one hour. After allowing the solution to cool, the mixture was quantitatively transferred to a 200 mL volumetric flask. The solution was diluted to 200 mL, mixed, and transferred to plastic storage vials (AOAC, 1984).

Ten mL samples of dissolved shell solution were added to 10 mL of distilled water and 10 mL of a 5 M potassium hydroxide-1 M potassium cyanide solution. This procedure raised the pH above 12 and allowed for the presence of only calcium to be tested by eliminating interference from other hardness metals especially magnesium. Approximately 35 mg of calcein indicator was added to produce a green color for titration analysis. The solution was titrated with a 0.4% EDTA standardized solution until a permanent purple color was observed.

Titration values for each shell were averaged over 3 trials, and used to calculate the amount of calcium (in mg) in the 10 mL sample, the total calcium in each shell tested, and the percentage of calcium per shell. MINITAB was used to conduct an analysis of variance and multiple comparison of means on the percentage values (arcsine transformed) to



determine any significant differences among test groups (Sokal & Rohlf, 1981).

### RESULTS

The eight groups of shells vary significantly in calcium content ( $F=10.75$ ,  $P<0.001$ ; Figure 2). Large control Lake Superior shells had significantly lower calcium levels than all other shells tested. Small and large control shells from Lake Erie did not differ significantly, but large test shells from Lake St. Claire had significantly less calcium than small test shells from Lake St. Claire. Small test Lake St. Claire shells, the small test Lake Erie shells in  $\text{CaCl}_2$ , and small test Lake St. Claire shells in Lake Superior water did not differ significantly.

### DISCUSSION

The analysis indicated a difference in the calcium content of the shells from the various Great Lakes. Despite these differences, the zebra mussels are able to survive and produce normal-appearing shells in all of the Great Lakes.

Environmental calcium concentrations could play a critical role in mollusk development given the importance of calcium in shell formation. The lack of significant differences among groups of small test shells seems to indicate that short-term (2.5 months) changes in environmental calcium concentrations have minimal effects on the calcium content in zebra mussel shells (Figure 2). Furthermore, the lack of a significant difference between

the large control Lake Erie and Lake Michigan shells suggests that small calcium differences of about 5 ppm do not significantly affect shell composition.

The lack of significant differences between the small test shells grown in water hardened with two different calcium compounds ( $\text{CaCO}_3$  and  $\text{CaCl}_2$ ) indicates no relationship between shell content and the initial compound used to create the calcium ion. This suggests that shell content is influenced by the calcium ions ( $\text{Ca}^{2+}$ ) present in the environment and not the associated anions.

The relationship between shell size and calcium content is less clear. Large control shells from Lake Erie of approximately 1.74 cm. had slightly less calcium relative to the small control shells. Larger test shells from Lake St. Claire averaging 2.01 cm. had an even lower calcium content compared to small test Lake St. Claire shells. This may suggest that the calcium content of zebra mussel shells undergoes a gradual reduction as the mussel grows in size. Therefore an inverse relationship may exist between shell size and calcium content of zebra mussel shells.

The significant differences between large control Lake Superior shells and those of the other Great Lakes appears to indicate that the low environmental calcium concentrations do affect shell composition. Although this difference does not prevent the zebra mussel from establishing itself in Lake Superior, the lower percent

calcium may signify a change in the zebra mussel's life history possibly resulting in a reduced growth rate, lower carrying capacity, or decreased survivorship.

Lower environmental calcium levels may induce additional stress to zebra mussels. A high energy expenditure may be needed to maintain a protective shell. This increased stress may weaken and reduce the overall vigor of the zebra mussel. The zebra mussels may have less energy available for growth and reproduction due in part to the calcium requirements of mussels for shell formation.

The lower calcium content in the zebra mussel shells from Lake Superior indicates a change in the overall composition of the shell which may be physically weaker and thus less protective. Zebra mussels growing under lower environmental levels of calcium may be more susceptible to predation, parasites, and diseases. This increase susceptibility may reduce the average life span of the mussel.

The mussels may only be capable of maintaining minimum growth rates to compensate for the low environmental calcium levels. Zebra mussels growing under low calcium conditions and with already low calcium contents would potentially continue to decrease the calcium content in their shells as they attempt to increase in size. The low levels of calcium in Lake Superior of 13 ppm may thereby limit the maximum size of the zebra mussel. The reduced size could

thereby make zebra mussels in Lake Superior more susceptible to predation by native fish and water fowl.

If true, then zebra mussels in Lake Superior may be less likely to displace the native mussel and clam populations. Although little is known about the native mollusc populations of Lake Superior (Hornbach, 1991), the native species may be able to more effectively outcompete the stressed zebra mussels in comparison to the native mussels in the other Great Lakes. The lower calcium levels may thus result in lower population densities of the zebra mussels in relationship to the native species.

Stanczykowska reported a significant relationship between environmental calcium levels and the population abundance of Dreissena polymorpha in Europe (1964).

Long-term exposure to lower environmental calcium concentrations affects the shell composition of the zebra mussel. The environmental calcium levels in conjunction with other environmental factors (water temperature) and ecological factors (competition and predation) may reduce the risk of a large zebra mussel population and the associated problems in Lake Superior that have been so characteristic of the other Great Lakes.

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# Zebra Mussel Infestation into the Great Lakes



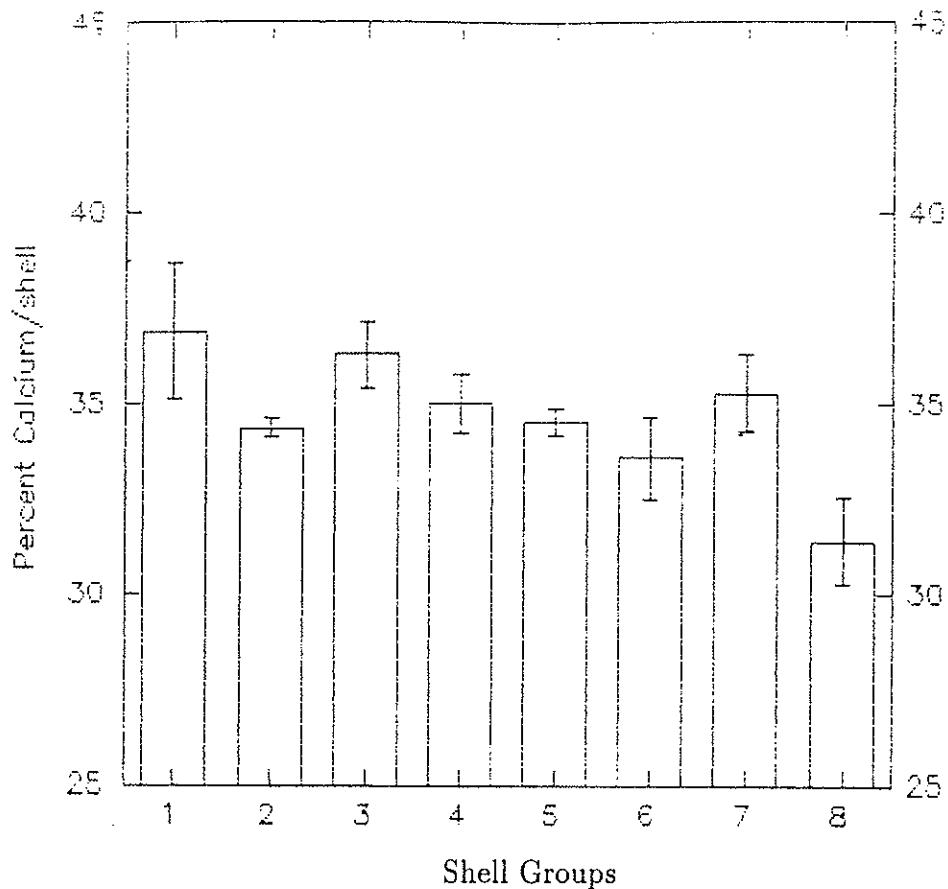
## First Reported Sightings of Zebra Mussels

1. Lake St. Claire (near Detroit, MI)- June, 1988
2. Lake Erie - July, 1988
3. Lake Ontario - November, 1989
4. St. Lawrence River - Spring, 1990
5. Lake Michigan - Spring, 1990
6. Lake Superior - April, 1990
7. Mississippi River (near La Crosse, WI) - September, 1991 (not shown)

(Figure 1 - The range of zebra mussels in North America as of February, 1991 are depicted by the darkened regions (Snyder, 1991). The quick spread of the mussel through the Great Lakes Region is illustrated in the sighting listing.)



# Differences in Shell Composition



## Shell Groups

1. Small Test St. Claire shells
2. Large Test St. Claire shells
3. Small Test Lake Erie shells in  $\text{CaCl}_2$
4. Small Control Lake Erie shells
5. Large Control Lake Erie shells
6. Large Control Lake Michigan shells
7. Small Test Lake St. Claire shells in Lake Superior Water
8. Large Control Lake Superior shells

(Figure 2 - The mean calcium content of shells from eight different natural and experimental waters with 95% confidence intervals error bars.)